JOURNAL OF ENDOUROLOGY
Volume 27, Number 10, October 2013

Pp. 1267–1271
DOI: 10.1089/end.2013.0234

Histotripsy Effects on the Bladder Trigone: Functional and Histologic Consequences in the Canine Model

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Abstract

Background: Histotripsy is an extracorporeal therapeutic ultrasound (US) technology, where high-amplitude acoustic energy is applied to targeted tissue. Previous research has demonstrated the feasibility, safety, and effectiveness of histotripsy tissue homogenization and debulking of the prostate in the canine model. Before translating this technology for human use, it is prudent to examine the susceptibility of critical periprostatic structures to cavitation injury in the event of histotripsy mistargeting. In this study, we sought to characterize the tissue effects and biologic response of directly treating the bladder trigone with histotripsy.

Materials and Methods: In eight anesthetized canines, 750,000 histotripsy pulses were applied uniformly across a 2×1.5-cm area encompassing the bladder trigone and ureteral orifices. Prostate and bladder trigone were harvested immediately after treatment (2 subjects) or at 14 days (6 subjects). Flexible cystourethroscopy, US imaging, and creatinine levels were obtained at intervals until harvest, 14 days after treatment. In one control subject, harvested at 2 days, the same treatment algorithm was applied to the prostate.

Results: Transrectal US imaging revealed a cavitation bubble cloud on the surface of the bladder trigone and progressive development of tissue edema during treatment. Flexible cystourethroscopy immediately after treatment confirmed edema and erythema of the trigone. In the six subjects survived 2 weeks after treatment, one incidence of transient, self-limited ureteral obstruction was noted based on hydronephrosis and creatinine levels. At harvest, ureteral orifices were confirmed patent by passage of a guide wire. Histologic evaluation revealed hemorrhage acutely with mild localized fibrosis at 14 days.

Conclusions: In this study, designed along the lines of a worst-case, destructive testing scenario, direct targeting of the bladder trigone with supratherapeutic histotripsy failed to induce significant tissue damage or clinical complication. These results are reassuring and will guide treatment strategy in upcoming human clinical trials of histotripsy treatment for benign prostatic hyperplasia.

Introduction

Histotripsy is an extracorporeal therapeutic ultrasound (US) technology, where application of very high-amplitude acoustic energy creates a cavitation bubble cloud within a geometric focal volume (a cigar-shaped 8 mm long by 3-mm-wide volume).[1] The acoustic pulses are short (<50 microseconds) and applied at a <1% duty cycle to minimize thermal tissue effects.[2] The microbubbles within the bubble cloud are transient. After each acoustic pulse, they exhibit dynamic oscillation and collapse, which transmit mechanical forces to the adjacent tissues and structures.[3,4] The bubble cloud appears hyperechoic (bright) on conventional diagnostic US and is confined to the focal volume where induced negative pressures exceed the cavitation threshold.[5]

Within predominantly glandular tissues (e.g., prostate parenchyma, liver, renal cortex), repetitive histotripsy pulses progressively homogenize cellular and architectural structures converting the targeted tissue into a pool of liquefied subcellular debris.[4,6] Clinically relevant volumetric ablation is accomplished by translating the bubble cloud throughout the desired targeted volume guided by real-time US imaging feedback.

Previous research has demonstrated the feasibility, safety, and effectiveness of histotripsy for ablation and debulking of the prostate in the canine model.[6] Before translating this technology for human therapy of benign prostatic hyperplasia (BPH), it is prudent to examine the susceptibility of critical periprostatic structures to cavitation injury in the event of histotripsy mistargeting. In an earlier study, the periprostatic neurovascular bundles and urinary sphincter were found to

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be resilient to structural damage even at supratherapeutic histotripsy doses (up to 100k pulses targeted on a single point).[7] The histotripsy threshold for cavitational injury to the rectum was lower such that collagen disruption and hemorrhage were seen when >10,000 histotripsy pulses were applied directly to the rectal wall.[7] In this study, we sought to characterize the tissue effects and biologic response of the bladder trigone and ureteral orifices to direct application of histotripsy therapy.

Materials and Methods

Nine intact male mongrel canines weighing 20–30 kg were used in this study after approval from the Institutional Animal Care and Use Committee. In preparation for histotripsy treatment, each subject was anesthetized using acepromazine (0.1 mg/kg) and intravenous propofol (2–8 mg/kg), intubated, and maintained with forced ventilation using isoflurane. The canines were given intramuscular Combi-Pen®(40,000 IU/kg), oral carprofen 2–4 mg/kg, and were administered a soap suds enema along with digital disimpaction after intubation. The abdomen and suprapubic region were shaved and depilated before positioning the canine supine on the procedural table.

Transrectal ultrasound (TRUS) imaging was performed using a Logiq P6 US scanner (GE Healthcare, Piscataway, NJ), which allowed visualization of the prostate and bladder base, including trigone. Flexible cystourethroscopy (8.2Fr Dur™-8 flexible ureteroscope) was performed in conjunction with TRUS to visualize the ureteral orifices and facilitate planning and histotripsy targeting.

The histotripsy transducer consisted of an 11×14-cm-diameter oval-shaped piezoelectric device (750 kHz, focal length 10 cm, and focal volume 3×3×8 mm, Imasonic, Inc., Voray-sur-l'Ognon, FR) suspended from a three-axis computer-controlled positioning system above the lower abdomen (Fig. 1). Coupling was accomplished from the extracorporeal transducer to the subject by means of a bolus of degassed water contained in a plastic membrane within a metal frame positioned on the canine suprapubic abdomen. Histotripsy pulses consisted of three-cycle bursts of acoustic energy (4 microseconds) delivered at a pulse repetition frequency of 500 Hz. Real-time TRUS imaging allowed identification and proper localization of the bubble cloud within the targeted region as well as monitoring of the bladder and trigone during treatment.

In each of the eight subjects, 750,000 pulses were applied uniformly across a 2-cm-wide (lateral) by 1.5-cm-long (cranial–caudal) area of the bladder trigone that encompassed both ureteral orifices. In one control subject, this same treatment pattern was applied to the prostate. Two subjects were harvested immediately after treatment and six were harvested on postprocedure day (POD) 14. The control subject was harvested on POD 2.

Daily veterinary evaluation was performed to assess for hematuria, urinary retention, pain, and changes in the dietary pattern or behavior. Flexible cystourethroscopy was performed at the completion of treatment and under anesthesia on POD 7 and 14 to visualize tissue responses to histotripsy. To assess for renal obstruction, bilateral renal US and serum creatinine were performed before treatment and on POD 2, 7, and 14.

At the time of harvest, the bladder and prostate were removed *en bloc* and grossly inspected. The bladder trigone (including the ureteral orifices) was placed in formalin for 1 week, and then dehydrated using 24% ethanol before hematoxylin and eosin and/or trichrome staining.

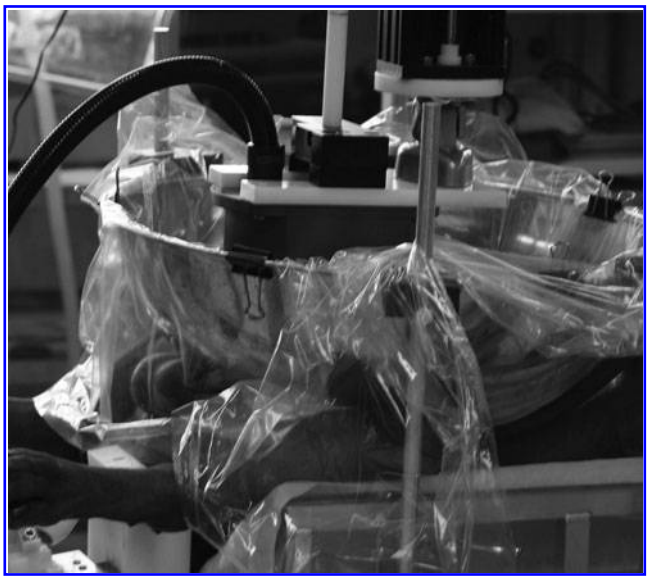

FIG. 1. Histotripsy therapy is delivered transabdominally from a piezoelectric transducer submerged in a degassed water bath, which provides coupling to the abdomen of a supine canine subject. Transrectal ultrasound (US) imaging provides high-resolution feedback of the histotripsy bubble cloud during treatment.

Results

In all subjects, TRUS imaging revealed a cavitation bubble cloud encompassing the surface of the bladder trigone during treatment (Fig. 2) and development of edema as treatment progressed. Flexible cystourethroscopy immediately after treatment confirmed edema associated with erythema of the trigone and a small volume of blood clot (Fig. 3A). In all, but one case, both ureteral orifices appeared to have been included in the treatment field.

Two subjects were harvested immediately after histotripsy treatment. In one, extensive multifocal hemorrhage in the lamina propria, smooth muscle, and surrounding adventitia was apparent just below the urothelial surface. At 1-mm depth, the lesion was mild, with a few small foci of intramuscular hemorrhage. There were no abnormalities in deeper sections. In the second subject, there was a single 12 mm^2 focus of hemorrhage in the trigonal muscle.

All six subjects harvested at 2 weeks did well clinically. They exhibited mild transient gross hematuria, but no urinary retention, abdominal distension, or other signs of distress. The initial mean creatinine level was 0.8 mg/dL (range 0.6 to 1.1 mg/dL) and exhibited a mild increase to 1.1 mg/dL (range 0.8 to 1.6 mg/dL) by POD 2 before returning to baseline at POD 7 (Fig. 4). New onset mild hydronephrosis was seen on renal US in one subject, which corresponded to a rise in creatinine to 1.6 mg/dL on POD 2. By POD 7, the unilateral hydronephrosis had resolved and creatinine had returned to baseline. No other subjects developed hydronephrosis. Flexible cystourethroscopy continued to reveal mild erythematous changes to the trigone until POD 14,

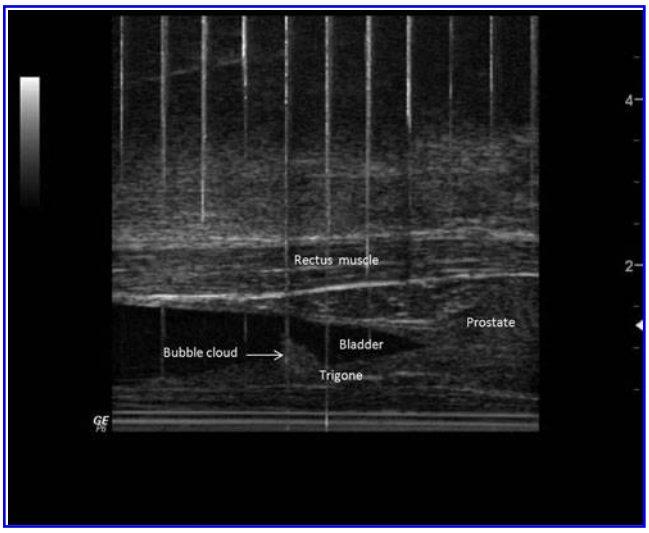

FIG. 2. Sagittal US image of a histotripsy bubble cloud on the surface of the trigone within the bladder during treatment.

when near-complete resolution was apparent (Fig. 3B). The ureteral orifices of all subjects were confirmed patent by passage of a guide wire easily into the ureter after harvest. Histologically, all hemorrhage had completely resolved by day 14. Five of six subjects exhibited minimal to mild, small ($<20\,mm^2$) multifocal, intramuscular, and superficial adventitial fibrosis revealed by trichrome staining. Two animals also had mild focal, mixed inflammation in the adventitia (Fig. 5). The total summation of histologic findings within the histotripsy treatment zone for each specimen was scored as 1 on a severity scale from 0–4. One subject exhibited a 2 mm erosion in the adjacent detrusor muscle accompanied by an area of granulation tissue with necrotic neutrophils at the surface and an underlying subacute inflammatory response.

In the control subject, the prostate volume was $43.2\,cm^3$ and the bubble cloud was easily followed as it was moved throughout the target volume within the prostate tissue and actually partially extended beyond the anterior prostate capsule. TRUS confirmed that a hypoechoic cavity had been created within the prostate. At the time of harvest, the prostate target volume was homogenized and largely drained via the urethra. Histologic evaluation confirmed that this treatment dose was sufficient to homogenize prostate tissue and create a large cavity defect consistent with results from prior work.

Discussion

The overarching goal of this research is to develop a histotripsy-based therapy for treatment of BPH. Histotripsy mechanically homogenizes prostatic tissue leaving a liquefied pool of debris, which can easily be passed in the urine. In this process, the anatomic result of transurethral resection of prostate could be realized with a potentially less morbid and more easily tolerated procedure. Much work has been completed in the canine model with regard to general feasibility, safety, and efficacy of the histotripsy therapy. As this technology approaches human clinical trials, effective treatment strategies can best be developed when the consequences of inadvertently treating adjacent structures beyond the prostate have been well evaluated. In this particular study, we purposely treated the bladder trigone and ureteral orifices with histotripsy doses much greater than would be needed for effective histotripsy therapy of the prostate.

Histotripsy effects on the bladder trigone are of particular interest as this technology is prepared for human use. In the canine model, histotripsy energy is delivered extracorporeally from a transducer positioned over the suprapubic region. The axis of energy delivery and the longest dimension of the bubble cloud are oriented in a ventral–dorsal direction. In the human, the best access to the prostate involves orienting the transducer such that the acoustic path traverses the perineum. In this scenario, the long access of the bubble cloud will be oriented along the cranial–caudal axis of the body. Treatment outside the prostate in this case is most likely to involve those structures just beyond the greatest extent of the bubble cloud (i.e., bladder trigone, ureteral orifices). Additionally, histotripsy treatment of adenoma in a median lobe may perhaps best be accomplished by incorporating a portion of the trigone and ureteral orifices in the target volume. It is well established that electrosurgical resection or laser vaporization of the bladder trigone and/or ureteral orifice can result in ureteral obstruction or stricture.[8] Furthermore, extensive urothelial denudation in and around the bladder neck is postulated to contribute to the formation of bladder neck contracture.[9]

Different tissue compositions require varying number of histotripsy pulses to produce tissue fractionation.[2,6] For

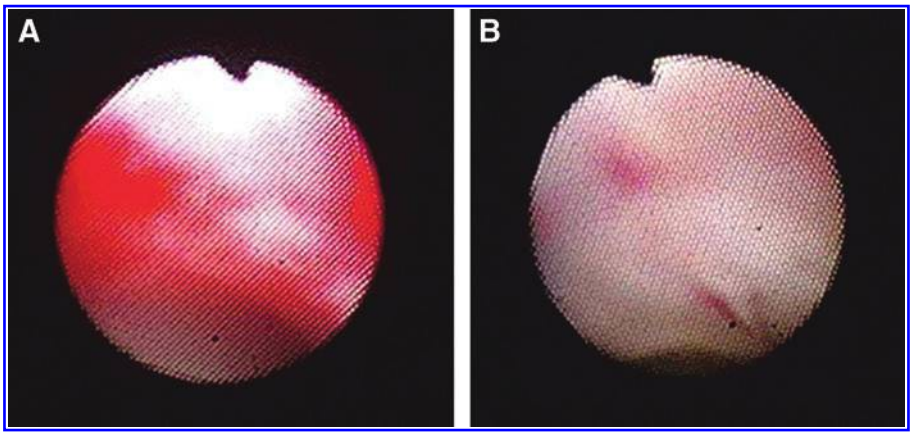

FIG. 3. **(A)** Endoscopic appearance of a canine trigone immediately following histotripsy treatment. Erythematous changes and a small area of hemorrhage are seen. **(B)** The same trigone, 14 days after histotripsy treatment, reveals resolution of erythema and hemorrhage.

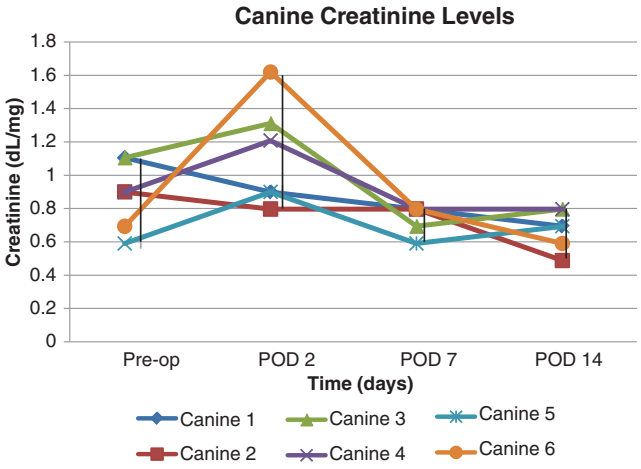

FIG. 4. Canine creatinine levels following histotripsy treatment of the bladder trigone.

example, in the prostatic urethra and periurethral stroma, a greater number of pulses are needed to achieve homogenization than in prostate glandular tissue.[10] Results from this study suggest that the bladder trigone also appears to have a high threshold for tissue homogenization. Although interstitial hemorrhage and surface edema was apparent immediately after treatment, these effects did not penetrate beyond 1 mm below the surface of the trigone. The resultant fibrosis that replaced the hemorrhage at 2 weeks was mild compared to the response after other known insults (i.e., trauma, localized inflammation) to the bladder trigone and is likely clinically insignificant.

These findings are consistent with previous research on histotripsy–tissue interactions and support the hypothesis that organs or structures containing closely packed fibers (e.g., muscles, nerves) may be less susceptible histotripsy bioeffects than glandular and epithelial structures, which contain fluid-filled spaces and a greater proportion of loose connective tissue.[6]

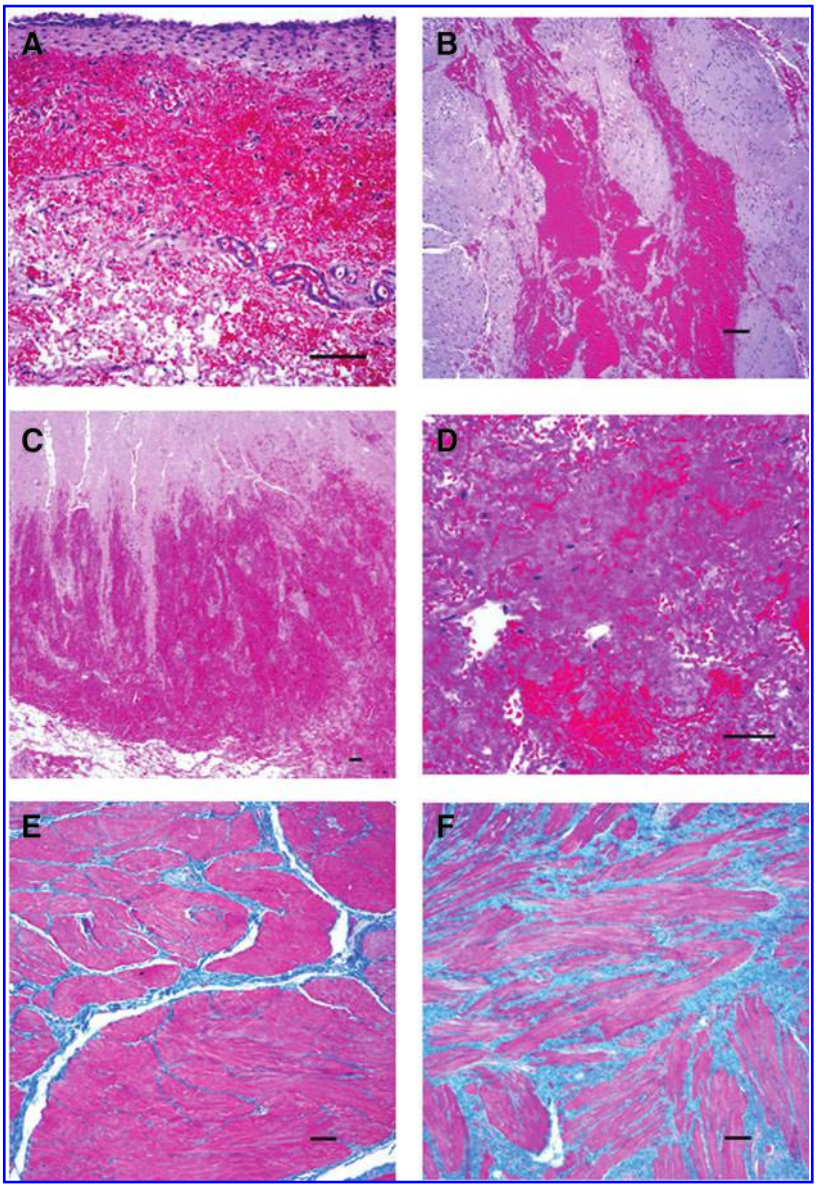

FIG. 5. **(A)** Focal hemorrhage in the adventitia of a bladder harvested immediately post-treatment (H&E). **(B)** Multifocal interstitial hemorrhage in the smooth muscle of a bladder harvested immediately post-treatment (H&E). **(C)** A single focus of hemorrhage in the interstitium of the smooth muscle of a bladder harvested immediately post-treatment (H&E). **(D)** Focal liquefaction necrosis and hemorrhage in a bladder harvested immediately post-treatment (H&E). **(E)** Normal bladder 1000 μm from the site of treatment 14 days post-treatment (trichrome). **(F)** A single focal area of marked fibrosis at the site of treatment 14 days post-treatment (trichrome). Such areas were rare. Scale bar = 50 μm.

Self-limited ureteral obstruction was suggested in only 1 of the 12 renal units, by renal US which demonstrated transient hydronephrosis. A lesser degree of obstruction likely occurred in several of the other subjects as suggested by a mild transient rise in creatinine, although the clinical significance of this is limited. Rapid resolution of the hydronephrosis, creatinine normalization, and patency of the ureteral orifice at harvest suggests no long-term deleterious effects and may, in fact, be similar to the clinical response after ureteral instrumentation with ureteroscopy.

Whereas the canine prostate is anatomically most similar to the human prostate, differences in location within the pelvis and tissue characteristics of the prostate do exist and may influence selection of optimal acoustic parameters. Furthermore, it is difficult to assess a subjective response to histotripsy treatment in the canine model such as irritative symptoms. Although transient effects of treatment resolve by 14 days, a longer assessment extending months beyond treatment is planned in initial human trials to assess for more delayed effects such as bladder neck contracture and scarring.

Conclusions

In this study, designed along the lines of a worst-case, destructive testing scenario, direct targeting of the bladder trigone with a supratherapeutic histotripsy dose failed to induce significant tissue damage or clinical complication. The typical response to supratherapeutic histotripsy was localized edema and superficial erythema. In only 1 of the 12 renal units was hydronephrosis observed on US, suggesting ureteral obstruction, which subsequently resolved without intervention. These results are reassuring and will guide treatment strategies as histotripsy treatment of BPH transitions to human clinical trials.

Acknowledgments

Funding: NIH R01 DK087871.

Author Disclosure Statement

Timothy L. Hall and William W. Roberts have consulting, equity, and royalty interests in HistoSonics, Inc.

Christopher L. Allam, J. Erby Wilkinson, Xu Cheng, and Kimberly A. Ives have no conflicts.

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Abbreviations Used

BPH = benign prostatic hyperplasia
POD = postprocedure day
TRUS = transrectal ultrasound
US = ultrasound

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